Appendix 8

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Immunogenicity and Safety of a Combination Pneumococcal-Meningococcal Vaccine in Infants

A Randomized Controlled Trial

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Context The success of conjugate vaccines in decreasing invasive disease due to *Streptococcus pneumoniae* and group C *Neisseria meningitidis* has placed pressure on crowded infant immunization schedules, making development of combination vaccines a priority.

Objective To determine the safety and immunogenicity of a combination 9-valent pneumococcal–group C meningococcal conjugate candidate vaccine (Pnc9-MenC) administered as part of the routine UK infant immunization schedule at ages 2, 3, and 4 months.

Design, Setting, and Participants Phase 2 randomized controlled trial conducted from August 2000 to January 2002 and enrolling 240 healthy infants aged 7 to 11 weeks from 2 UK centers, with home follow-up visits at ages 2, 3, 4, and 5 months.

Intervention Pnc9-MenC (n=120) or monovalent group C meningococcal conjugate vaccine (MenC) (n=120) administered in addition to routine immunizations (diphtheria and tetanus toxoids and whole-cell pertussis [DTwP], *Haemophilus influenzae* type b [Hib] polyribosylribitol phosphate-tetanus toxoid protein conjugate, oral polio vaccine).

Main Outcome Measures Group C meningococcal immunogenicity measured by serum bactericidal titer (SBT) 1 month following the third dose; rates of postimmunization reactions.

Results MenC component immunogenicity was reduced in the Pnc9-MenC vs the MenC group (geometric mean SBT, 179 [95% confidence interval (CI), 133-243] vs 808 [95% CI, 630-1037], respectively; $P<.001$). The proportion with group C meningococcal SBT greater than 1:8 was lower in the Pnc9-MenC vs the MenC group (95% vs 100%, $P=.05$). The geometric mean concentration of antibodies to concomitantly administered Hib vaccine was reduced in the Pnc9-MenC vs the MenC group (2.11 [95% CI, 1.57-2.84] µg/mL vs 3.36 [95% CI, 2.57-4.39] µg/mL; $P=.02$), as were antibodies against diphtheria (0.74 [95% CI, 0.63-0.87] µg/mL vs 1.47 [95% CI, 1.28-1.69] µg/mL; $P<.001$). Pnc9-MenC was immunogenic for each of 9 contained pneumococcal serotypes, with responses greater than 0.35 µg/mL observed in more than 88% of infants. Increased irritability and decreased activity were observed after the third dose in the Pnc9-MenC group.

Conclusions Pnc9-MenC combination vaccine administered to infants at ages 2, 3, and 4 months demonstrated reduced group C meningococcal immunogenicity compared with MenC vaccine. The immunogenicity of concomitantly administered Hib and DTwP vaccines was also diminished. The Pnc9-MenC vaccine was safe and immunogenic for all contained pneumococcal serotypes. The reduced MenC immunogenicity may limit the development of the Pnc9-MenC vaccine.

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THE DEVELOPMENT OF IMMUNOgenic vaccines against major encapsulated pathogens of childhood has dramatically decreased the rates of invasive disease caused by *Haemophilus influenzae* type b, serogroup C *Neisseria meningitidis*, and *Streptococcus pneumoniae* in the developed countries fortunate enough to be able to afford the vaccines. Since the introduction of a monovalent serogroup C meningococcal glycoconjugate vaccine (MenC) into the UK routine immunization schedule in November 1999, group C meningococcal disease has decreased by 87% in the ages targeted for vaccination, with estimated vaccine efficacy of 90%.[1] Within 2 years of the introduction of a 7-valent pneumococcal glycoconjugate vaccine into the recommended infant schedule in the United States, there was

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a 69% reduction in culture-positive invasive pneumococcal disease in children younger than 2 years.[2] However, outside North America, and especially in developing nations where the toll of pneumococcal disease is highest, the 7-valent pneumococcal glycoconjugate vaccine does not cover all of the prevalent serotypes. In particular, the inclusion of serotype 1 is considered vital to achieve optimal coverage internationally.

The advent of these efficacious and safe vaccines has increased pressure on crowded infant immunization schedules. Infants in the United States receive up to 20 separate vaccine injections over 5 immunization encounters at ages 2, 4, 6, 12, and 18 months to protect against disease due to hepatitis B, diphtheria, tetanus, pertussis, polio, measles, mumps, rubella, varicella, *H influenzae* type b, and pneumococcus. The UK immunization schedule differs in having an accelerated infant schedule at ages 2, 3, and 4 months and no 18-month booster visit. Infants in the United Kingdom also do not receive hepatitis B, varicella, or pneumococcal vaccine, but routinely do receive 3 doses of MenC vaccine. The inclusion of MenC would add a further 3 to 4 doses to the US regimen, while the inclusion of pneumococcal glycoconjugate vaccine would add at least 3 doses for UK infants. Many other countries, including Australia, Canada, and western European nations, have incorporated MenC and/or pneumococcal glycoconjugate vaccines into their recommended schedules.

In anticipation of the recommendation of both these vaccines in many developed countries, the development of combination vaccines has become a priority. The combining of pneumococcal and meningococcal conjugate vaccines has the potential to spare US infants up to 4 extra injections by age 18 months and to decrease parental and clinician concerns about the number of vaccinations in early childhood. In the United Kingdom, where vaccines are routinely administered in just 2 syringes at each vaccination visit, a combination meningococcal-pneumococcal vaccine would avoid the need to increase from 2 to 3 injections at each visit and thereby make the addition of pneumococcal vaccine to the schedule more acceptable. This report describes the immunogenicity and safety of a combination 9-valent pneumococcal–group C meningococcal polysaccharide-protein conjugate candidate vaccine (Pnc9-MenC) given to UK infants at ages 2, 3, and 4 months.

METHODS

Participants and Recruitment

The current study was a phase 2 randomized controlled trial conducted from August 2000 to January 2002 and enrolling healthy infants aged 7 to 11 weeks from 2 centers in Oxford and Gloucester, United Kingdom. In Oxford, up to 2 letters were sent to parents of eligible infants belonging to general practice lists of 16 participating practices in Oxfordshire, inviting them to contact the investigators regarding the study. Parents interested in the study were visited at home by research nurses. If families wished to participate, informed written consent was obtained, followed by additional home visits. In Gloucester, parents of eligible children in the practice were sent letters of invitation to participate, and parents of eligible infants who expressed an interest in the study were contacted by research nurses. Written consent was obtained at 1 of 14 participating practices, and subsequent visits also occurred there. Exclusion criteria included known immunosuppression, previous vaccination (apart from BCG or hepatitis B vaccines), and confirmed invasive meningococcal or pneumococcal disease. The study was approved by the Central Oxford Research Ethics Committee (COREC 00.043) and the Gloucestershire Local Research Ethics Committee (GLREC 99/1049).

Visits and Vaccines

The infants were visited at ages 2, 3, 4, and 5 months, with a window of 28 to 42 days between visits. Infants were immunized at 2, 3, and 4 months with diphtheria and tetanus toxoids and whole-cell pertussis (DTwP) vaccine (Aventis, Lyon, France), 0.5 mL, admixed with *H influenzae* type b (Hib) polyribosylribitol phosphate-tetanus toxoid protein conjugate (ActHib; Aventis), 0.5 mL, administered intramuscularly in the right anterolateral thigh. Infants also received oral polio vaccine, 2 drops administered orally. Infants were randomized in a 1:1 ratio at study entry to receive in their left anterolateral thigh either Pnc9-MenC (Wyeth Vaccines, Maidenhead, UK), 0.5 mL intramuscularly, or MenC (Meningitec; Wyeth Vaccines), 0.5 mL intramuscularly, at each vaccine visit. Randomization was performed by a computer-generated randomization list in blocks of 6. The study was not blinded because the Pnc9-MenC (lyophilized) and MenC (preprepared in the syringe) vaccines were visually different.

The Pnc9-MenC vaccine was a lyophilized preparation supplied in single-dose vials. Each 0.5-mL dose contained 2 µg of pneumococcal saccharide conjugates 1, 4, 5, 9V, 14, 18C, 19F, and 23F; 4 µg of pneumococcal saccharide conjugate 6B; 10 µg of meningococcal group C oligosaccharide; and approximately 38.5 µg of cross-reacting material 197 (CRM_{197}) carrier protein (a nontoxic variant of diphtheria toxin) with 0.5 mg of aluminum phosphate (0.125 mg elemental aluminum) as adjuvant. The MenC is a conjugate vaccine containing meningococcal serogroup C oligosaccharide conjugated to CRM_{197}. Each 0.5-mL dose contained 10 µg of meningococcal group C oligosaccharide, 15 µg of CRM_{197} carrier protein, and 0.5 mg of aluminum phosphate (0.125 mg elemental aluminum) as adjuvant. The MenC component in the Pnc9-MenC vaccine was derived from a different manufacturer lot than that in the MenC vaccine. Venipuncture was performed at the 2- and 5-month visits, and 2.5 to 5.0 mL of blood was obtained for serologic assays.

Safety Evaluation

Following each immunization visit, infants were observed for 20 minutes for immediate hypersensitivity reactions, and parents completed diaries for the day of immunization and the 3 following days, recording all local and systemic adverse events. Local reactions solicited included tenderness and presence and size of erythema and induration. Systemic events solicited included drowsiness, change in appetite, irritability, persistent crying, changes in activity level, and use of antipyretic medication. Parents measured their child's axillary temperature on the evening after vaccination and the subsequent 3 evenings. Fever at any other time in the 4-day period was also recorded. Information on adverse events was actively obtained by research nurses using telephone calls up to twice in the week after vaccination and monthly visits up to age 5 months (1 month after the last vaccination). All events requiring a visit to a clinician, as well as all serious adverse events, were reported. Temperature greater than 39.5°C per axilla within 48 hours and prolonged inconsolable or high-pitched screaming for more than 3 hours following vaccination were exclusion criteria for further study doses.

Serologic Assays

Serum samples were tested by serum bactericidal assay (SBA) for functional antibody against group C *N meningitidis* as previously described, using baby rabbit complement, at the Public Health Laboratory Service Meningococcal Reference Unit, Manchester, UK.[3] SBA titers were expressed as the reciprocal serum dilution yielding 50% or greater killing after 90 minutes. SBA titers less than 4 were designated a value of 2. The geometric mean titer (GMT) and the proportion in each group exceeding titers of the protective correlate of 1:8 and the suggested long-term protected correlate of 1:128 were determined for each group. Enzyme-linked immunosorbent assays (ELISAs) were also performed for antibody responses to meningococcal group C capsular polysaccharide, diphtheria, tetanus, pertussis (pertussis toxin, filamentous hemagglutinin), and Hib as previously described.[4] For diphtheria and tetanus, the accepted protective correlate of 0.1 µg/mL was used, while for Hib the short-term (0.15 µg/mL) and long-term (1.0 µg/mL) correlates were used.[5] Polio types 1, 2, and 3 responses were assessed by neutralization assay.[4] Geometric mean concentrations (GMCs) were determined for each group, and the proportions reaching predetermined protective cutoff levels for each assay were also described.

Pneumococcal serotype-specific antibody concentrations were measured using IgG ELISAs to detect antibody to pneumococcal capsular polysaccharides types 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F.[6] A number of protective cutoffs have been suggested, with 0.2 µg/mL and 0.35 µg/mL most often proposed as short-term protective correlates. Of these, the more stringent 0.35 µg/mL was used in this study, but 0.2 µg/mL is also presented. The long-term correlate used was 1.0 µg/mL.

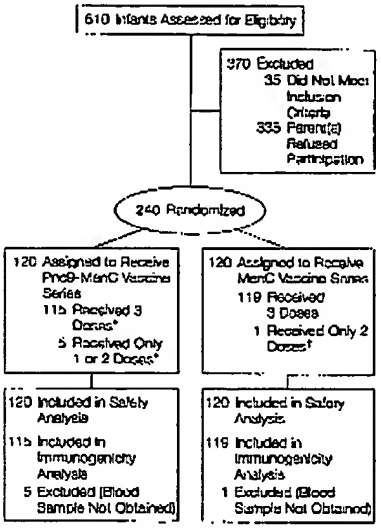
All assays were performed at Wyeth Laboratories, Rochester, NY, apart from SBAs against group C *N meningitidis*. Laboratory staff were blinded to participant allocation.

Statistical Analyses

Antibody levels were log-transformed, and GMTs and GMCs with 95% confidence intervals (CIs) calculated. The *t* test was used to compare postvaccination GMTs (MenC SBA) or GMCs between groups. The χ^2 test was used to compare proportions achieving predetermined protective levels between groups. The χ^2 test was also used to compare proportions of infants with adverse reactions between groups.

The predicted sample was based on the primary immunogenicity measure of meningococcal group C SBA after dose 3 for a range of threshold values from 1:8 to 1:256. Based on previous data showing that more than 95% of infants in the MenC control group had SBTs greater than 1:8, 100 per group were required to demonstrate noninferiority of Pnc9-MenC, with α=.05 and power of 80%.[7] Allowing for a 20% dropout rate, a sample size of 240 infants was selected. Analyses were performed using Intercooled Stata version 8.0 (Stata Corp, College Station, Tex).

Figure. Flow Diagram of Study Groups



MenC indicates monovalent group C meningococcal conjugate; Pnc9-MenC, 9-valent pneumococcal–group C meningococcal conjugate

*Two infants withdrawn following first vaccination, 1 due to fever and 1 due to prolonged crying and fever; 3 infants withdrawn following second vaccination (2 due to prolonged crying, 1 due to fever).

†Withdrawn following second vaccination due to prolonged crying.

RESULTS

Two hundred forty infants were randomized between August 2000 and September 2001. The Pnc9-MenC group contained 66 male and 54 female infants of mean age 1.96 months; the MenC group contained 63 male and 57 female infants of mean age 1.92 months. The participant flow is summarized in the FIGURE.

Safety

Both Pnc9-MenC and MenC were well tolerated, with no immediate adverse events. Two infants in the Pnc9-

MenC group were withdrawn following the first vaccination at 2 months, 1 due to fever and 1 due to prolonged crying and fever. Four infants were withdrawn after the second vaccination at 3 months, 3 due to prolonged crying (2 Pnc9-MenC, 1 MenC) and 1 due to fever (Pnc9-MenC). TABLE 1 describes the proportions in each group experiencing local and systemic adverse events. Local reactions in the study vaccine limb were uncommon in both groups. Temperature greater than 38°C per axilla was reported in 42 infants (35.3%) receiving Pnc9-MenC and in 32 (26.7%) of those receiving MenC ($P=.15$). Irritability (65.2% vs 48.7%, $P=.02$) and decreased activity (33.0% vs 20.2%, $P=.03$) were reported more frequently after the third dose in the Pnc9-MenC group than in the MenC group, but not after other doses.

Immunogenicity

The Pnc9-MenC vaccine was less immunogenic than the MenC vaccine in induction of serogroup C meningococcal polysaccharide antibodies as determined at age 5 months by both SBA and ELISA (SBA GMT, 179 [95% CI, 133-243] for Pcn9-MenC vs 808 [95% CI, 630-1037] for MenC; $P<.001$) (TABLE 2). The proportions of infant serum samples with serogroup C meningococcal SBA titers greater than 1:128 and greater than 1:8 were also lower in this group.

Geometric mean Hib antibody concentrations following 3 doses of Hib vaccine were also reduced in the Pnc9-MenC group when compared with the MenC group (2.11 [95% CI, 1.57-2.84] µg/mL vs 3.36 [95% CI, 2.57-4.39] µg/mL; $P=.02$) (Table 2). However, the lower GMC was not reflected in differences in the proportions of serum samples in the 2 groups exceeding 0.15 µg/mL and 1.0 µg/mL at 5 months of age. Antidiphtheria antibody levels also differed between the groups when measured at 5 months, with lower GMC achieved by the Pnc9-MenC group when compared with the MenC group (0.74 [95% CI, 0.63-0.87] µg/mL vs 1.47 [95% CI, 1.28-1.69] µg/mL, respectively; $P<.001$) (Table 2), although all infants in both groups surpassed the protective cutoff of 0.1 µg/mL. Antitetanus GMCs did not differ significantly. There were no differences between the groups in responses to the other concomitantly administered vaccine antigens at age 5 months following the 3-dose routine primary vaccine series.

All pneumococcal serotypes contained in the vaccine were immunogenic (TABLE 3), with at least 88% of recipients developing postvaccination GMCs greater than 0.35 µg/mL to each serotype. The greatest proportions developing GMCs greater than 1.0 µg/mL were achieved for serotypes 1, 4, 14, and 19F and the lowest for serotypes 6B and 9V.

COMMENT

This study describes the first infant trial of a candidate combination Pnc9-MenC conjugate vaccine. It illustrates the unpredictability of immunogenicity when combining multivalent vaccines, each immunogenic in separate form. The reduced immunogenicity of the serogroup C meningococcal component of Pnc9-MenC as well as concomitantly administered Hib and diphtheria may limit its further development.

We found Pnc9-MenC to be well tolerated and immunogenic for the pneumococcal serotypes contained in the vaccine. While absolute pneumococcal serotype-specific serologic corre-

Table 1. Reactogenicity of Pnc9-MenC and MenC Vaccines Given Concurrently With DTwP/Hib and OPV Vaccines in Infants Aged 2, 3, and 4 Months

	Events, No. (%)					
	First Dose		Second Dose		Third Dose	
Adverse Event	Pnc9-MenC (n = 120)	MenC (n = 120)	Pnc9-MenC (n = 118)	MenC (n = 120)	Pnc9-MenC (n = 115)	MenC (n = 119)
Local reactions						
Erythema	2 (1.7)	0	0	0	0	1 (0.8)
Swelling	1 (0.8)	1 (0.8)	1 (0.8)	0	0	1 (0.8)
Tenderness	3 (2.5)	5 (4.2)	1 (0.8)	1 (0.8)	0	2 (1.7)
Any local reaction	8 (6.7)	11 (9.2)	9 (7.6)	15 (12.5)	6 (5.2)	10 (8.4)
Systemic reactions						
Fever (temperature >38°C)	21 (17.5)	15 (12.5)	10 (8.5)	5 (4.2)	14 (12.2)	9 (7.6)
Irritability (any)	92 (76.7)	98 (81.7)	83 (70.3)	78 (65.0)	75 (65.2)*	58 (48.7)
Increased crying	3 (2.5)	4 (3.3)	3 (2.5)	3 (2.5)	3 (2.6)	1 (0.8)
Drowsiness	81 (67.5)	73 (60.8)	68 (57.6)	58 (48.3)	60 (52.2)	48 (40.3)
Decreased activity	50 (41.7)	50 (41.7)	44 (37.3)	35 (29.2)	38 (33.0)†	24 (20.2)
Anorexia	55 (45.8)	49 (40.8)	38 (32.2)	37 (30.8)	32 (27.8)	26 (21.8)
Any systemic reaction	108 (90.0)	110 (91.7)	102 (86.4)	95 (79.2)	91 (79.1)‡	79 (66.4)

Abbreviations: DTwP, diphtheria and tetanus toxoids and whole-cell pertussis; Hib, *Haemophilus influenzae* type b; MenC, monovalent group C meningococcal conjugate; OPV, oral polio vaccine; Pnc9-MenC, 9-valent pneumococcal-group C meningococcal conjugate.
*$P = .02$ for χ^2 test of proportions between groups.
†$P = .03$ for χ^2 test of proportions between groups.
‡$P = .03$ for χ^2 test of proportions between groups.

lates of protection are unknown, at least 88% of infants achieved antibody concentrations of 0.35 µg/mL or greater 1 month after their third dose for each serotype.[7] The immunogenicity results for the serotypes also contained in the licensed 7-valent pneumococcal conjugate Prevnar (Wyeth Vaccines) (4, 6B, 9V, 14, 18C, 19F, and 23F) are similar to those obtained after 3 doses at ages 2, 4, and 6 months with that vaccine.[8] Good immunogenicity was also achieved for the additional serotype 1, but relatively lower GMCs were found for serotype 5, as observed with other 9- and 11-valent pneumococcal vaccines using varying conjugating proteins.[9-11]

The laboratory correlate of natural protection against invasive meningococcal group C disease was established during military recruit studies in the 1960s. Goldschneider et al[12] found the functional assay of group C meningococcal SBA titer to correspond with clinical protection, with a titer greater than 1:8 protective. Enzyme-linked immunosorbent assay levels of antibody to meningococcal group C capsular polysaccharide were found to correspond with this assay, with the protective cutoff at 2 µg/mL.[4] These assay levels formed the basis on which glycoconjugate MenC vaccines were licensed and introduced into the UK national immunization schedule in the absence of efficacy data. Similar protective levels of antibody to *H influenzae* type b were also accepted following polysaccharide vaccine studies, with a postvaccination level greater than 0.15 µg/mL corresponding to short-term protection and greater than 1.00 µg/mL with long-term protection. Laboratory correlates of protection against pneumococcal disease have yet to be agreed upon.

The Pnc9-MenC group achieved lower SBA titers to group C meningococcus than the MenC group, with a lower GMT. Fewer in the Pnc9-MenC group exceeded the protective threshold titer of 1:8 and the putative threshold titer of 1:128. Geometric mean concentrations of anticapsular polysaccharide antibody measured by ELISA were also lower in the Pnc9-MenC group, and fewer achieved the putative protective threshold of 2 µg/mL. The incorporation of different polysaccharide antigens into a multivalent conjugate vaccine is technically difficult and can unpredictably affect the immunogenicity of individual components. Previous hypotheses for unexpectedly low immunogenicity of conjugate vaccines have included carrier-induced epitope suppression, with immune responses directed against the carrier protein (here, the mutant diphtheria toxoid CRM_{197}) suppressing the response to the covalently bound polysaccharide.[13] The increased amount of carrier protein in each Pnc9-MenC dose (38 µg vs 10 µg in MenC) offers some support to this theory. At least 2 manufacturers have used mixed carrier proteins for candi-

Table 2. Immunogenicity of the MenC Vaccine Component and Concomitant Vaccines 1 Month After a 3-Dose Immunization Series*

Antigen/Assay	No.†	Pnc9-MenC	No.†	MenC	P Value
Neisseria meningitidis serogroup C					
SBA					
GMT (95% CI)	111	179 (133-243)	117	606 (630-1037)	<.001
Titer >1:8, No. (%)	115	109 (95)	118	117 (99)	.05
Titer >1:128, No. (%)	115	93 (81)	118	111 (94)	.002
ELISA					
GMC, µg/mL (95% CI)	115	4.68 (4.19-5.70)	116	23.27 (20.18-26.84)	<.001
GMC >2 µg/mL, No. (%)	115	105 (91)	118	118 (100)	.001
Hib ELISA					
GMC, µg/mL (95% CI)	102	2.11 (1.57-2.84)	108	3.36 (2.57-4.39)	.02
>0.15 µg/mL, No. (%)	115	110 (96)	118	115 (97)	.45
>1.00 µg/mL, No. (%)	115	86 (75)	118	100 (85)	.06
Tetanus ELISA					
GMC, µg/mL (95% CI)	99	4.65 (3.91-5.52)	105	5.69 (4.99-6.96)	.05
>0.1 IU/mL, No. (%)	115	115 (100)	118	118 (100)	
Diphtheria ELISA					
GMC, µg/mL (95% CI)	106	0.74 (0.63-0.87)	111	1.47 (1.28-1.69)	<.001
>0.1 IU/mL, No. (%)	115	115 (100)	118	118 (100)	
Bordetella pertussis ELISA GMC, µg/mL (95% CI)					
Pertussis toxin	102	24.36 (17.25-34.39)	110	28.89 (20.73-40.25)	.48
FHA	103	59.04 (51.41-67.80)	109	54.77 (47.69-67.80)	.44
Poliovirus GMC, µg/mL (95% CI)					
Poliovirus 1	101	101.4 (76.9-130.2)	102	86.3 (66.1-112.2)	.38
Poliovirus 2	101	240.7 (203.3-284.9)	102	245.8 (209.0-297.6)	.87
Poliovirus 3	101	116.3 (89.0-151.9)	102	112.5 (89.8-140.9)	.85
Streptococcus pneumoniae ELISA GMC by serotype, µg/mL (95% CI)					
1	100	1.43 (1.15-1.79)	104	0.09 (0.06-0.11)	<.001
4	100	1.20 (0.95-1.53)	104	0.04 (0.03-0.05)	<.001
5	100	0.77 (0.66-0.90)	104	0.23 (0.19-0.27)	<.001
6B	100	1.20 (0.95-1.52)	104	0.18 (0.14-0.22)	<.001
9V	100	0.94 (0.76-1.15)	104	0.12 (0.10-0.15)	<.001
14	100	2.49 (1.92-3.21)	104	0.11 (0.08-0.16)	<.001
18C	100	0.97 (0.79-1.20)	104	0.11 (0.09-0.14)	<.001
19F	100	1.83 (1.50-2.22)	104	0.28 (0.22-0.35)	<.001
23F	100	1.15 (0.93-1.43)	104	0.11 (0.08-0.13)	<.001

Abbreviations: CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; GMT, geometric mean titer; Hib, *Haemophilus influenzae* type b; MenC, monovalent group C meningococcal conjugate; Pnc9-MenC, 9-valent pneumococcal–group C meningococcal conjugate; SBA, serum bactericidal activity.

*GMT/GMC values compared between groups using *t* test. Proportions above serologic cutoffs compared between groups using χ^2 test.

†Number of infants with serum samples obtained.

date conjugate vaccines to lessen the impact of immune responses to carrier protein.[9,14] The MenC component of the Pnc9-MenC vaccine did come from a different manufacturer lot than the MenC used in the control group. While it is possible for minor immunogenicity differences to occur, it is unlikely that this is responsible for the observed difference, considering the additional effects on Hib and diphtheria immunogenicity in the Pnc9-MenC group.

Haemophilus influenzae type b antibody responses were also unexpectedly diminished in the Pnc9-MenC group. The Hib vaccine used in this trial was administered concurrently with the study vaccines in the opposite limb, admixed with DTwP. This reduction was not matched by reduced proportions achieving short-term protective titers greater than 0.15 µg/mL.[5] Fewer in the Pnc9-MenC group reached long-term protective titers greater than 1.00 µg/mL; however, the difference in proportions between groups was not significant. Similar reductions have been described when combination vaccines containing diphtheria-tetanus-acellular pertussis and Hib have been evaluated.[15] While the Hib conjugate vaccine used in the current study was coadministered in the same syringe as DTwP vaccine, it was administered at a separate site than the Pnc9-MenC or MenC vaccines, ruling out any physicochemical interference. Neither can the phenomenon of carrier-induced epitope suppression explain this finding, as Pnc9-MenC and MenC vaccines both use the mutant diphtheria toxoid CRM_{197} as their conjugate protein.[16] Nonepitope-specific suppression has been described in adults receiving Hib conjugate vaccines. Barington et al[16] described reductions in both Hib and tetanus toxoid antibody titers in patients receiving Hib-tetanus toxoid glycoconjugate vaccine who had been prevaccinated with Hib-diphtheria toxoid glycoconjugate vaccine 28 days earlier, compared with those who had not been prevaccinated. The same effect on Hib and diphtheria toxoid antibody titers was observed among patients prevaccinated with Hib-tetanus toxoid who were receiving Hib-diphtheria toxoid.

The impact on Hib vaccine immunogenicity is of particular interest in the United Kingdom, given the observed increase there in Hib vaccine failures in recent years. Rapid waning of initial titers has been described for all glycoconjugate vaccines, and a lower initial serologic response may result in more rapid decline in titers.[4,17] Similarly, recent analysis of the effectiveness of the national MenC vaccination program in the United Kingdom has identified a potential lack of protection beyond 1 year after vaccination for those vaccinated as infants (at ages 2, 3, and 4 months), despite immunological memory being demonstrated with this regimen.[18] In this context, a combination vaccine offering lower initial group C meningococcal immunogenicity would be unlikely to be acceptable. These issues are likely to be of particular concern where booster doses are not part of the immunization schedule. It is not known whether similar ef-

Table 3. Infants Achieving Putative Protective Pneumococcal Serotype-Specific Antibody Concentrations 1 Month After Primary Immunization Series*

		% (95% CI)					
		GMC ≥0.2 µg/mL		GMC ≥0.35 µg/mL		GMC ≥1.0 µg/mL	
Serotype	Age, mo	Pnc9-MenC	MenC	Pnc9-MenC	MenC	Pnc9-MenC	MenC
1	2	69.0 (59.8 to 76.2)	57.1 (47.5 to 66.8)	47.0 (37.0 to 57.0)	34.3 (25.1 to 43.5)	13.0 (6.3 to 19.7)	11.4 (5.2 to 17.6)
	5	94.0 (89.3 to 98.7)	22.2 (14.0 to 30.2)	92.0 (86.6 to 97.4)	15.4 (8.3 to 22.4)	79.0 (70.9 to 87.1)	4.8 (0.6 to 9.0)
4	2	38.0 (28.3 to 47.7)	34.3 (25.1 to 43.5)	21.0 (12.9 to 29.1)	17.1 (9.8 to 24.5)	3.0 (−0.4 to 6.4)	4.7 (0.6 to 8.9)
	5	93.0 (87.9 to 98.1)	14.4 (7.6 to 21.3)	92.0 (86.6 to 97.4)	8.7 (3.2 to 14.1)	76.0 (67.5 to 84.5)	4.8 (0.6 to 9.0)
5	2	88.0 (81.5 to 94.4)	83.8 (76.6 to 91.0)	76.0 (67.5 to 84.5)	70.5 (61.6 to 79.3)	27.0 (18.1 to 35.9)	19.0 (11.4 to 26.7)
	5	95.0 (90.7 to 99.3)	55.8 (46.1 to 65.5)	88.0 (81.5 to 94.5)	26.9 (18.3 to 35.6)	39.0 (29.3 to 48.7)	2.9 (−0.3 to 6.2)
6B	2	87.0 (80.3 to 93.7)	82.9 (75.5 to 90.2)	76.0 (67.5 to 84.5)	67.6 (58.5 to 76.7)	42.0 (32.2 to 51.8)	30.5 (21.5 to 39.4)
	5	95.0 (90.7 to 99.3)	40.4 (30.8 to 50.0)	89.0 (82.8 to 95.2)	24.0 (15.7 to 32.4)	58.0 (48.2 to 67.8)	7.7 (2.5 to 12.9)
9V	2	79.0 (70.9 to 87.1)	73.3 (64.7 to 81.9)	56.0 (46.1 to 65.9)	48.6 (38.9 to 58.3)	13.0 (6.3 to 19.7)	15.2 (8.2 to 22.2)
	5	93.0 (87.9 to 98.1)	29.8 (20.9 to 38.7)	89.0 (82.8 to 95.2)	17.3 (9.9 to 24.7)	54.0 (44.1 to 63.9)	3.8 (0.1 to 7.6)
14	2	70.0 (60.9 to 79.1)	66.7 (57.5 to 75.8)	64.0 (44.1 to 63.9)	52.4 (42.7 to 62.1)	39.0 (29.3 to 48.7)	27.6 (18.9 to 36.3)
	5	96.0 (92.1 to 99.9)	35.6 (26.2 to 44.9)	93.0 (87.9 to 98.1)	20.2 (12.3 to 28.0)	81.0 (73.2 to 88.8)	13.5 (6.8 to 20.1)
18C	2	73.0 (64.1 to 81.9)	68.6 (59.5 to 77.6)	48.0 (38.0 to 58.0)	46.7 (37.0 to 56.4)	15.0 (7.8 to 22.1)	16.2 (9.0 to 23.3)
	5	93.0 (87.9 to 98.1)	27.9 (19.1 to 36.6)	90.0 (84.0 to 96.0)	11.5 (5.3 to 17.8)	59.0 (49.2 to 68.8)	5.8 (1.2 to 10.3)
19F	2	89.0 (82.8 to 95.2)	89.5 (83.6 to 95.5)	80.0 (72.0 to 88.0)	77.1 (69.0 to 85.3)	49.0 (31.0 to 59.0)	38.1 (28.7 to 47.5)
	5	97.0 (93.6 to 100.0)	56.7 (47.0 to 66.4)	92.0 (86.6 to 97.4)	37.5 (28.0 to 47.0)	85.0 (77.9 to 92.1)	21.2 (13.2 to 29.1)
23F	2	74.0 (65.3 to 82.7)	71.4 (62.6 to 80.2)	60.0 (50.2 to 69.8)	45.7 (36.0 to 55.4)	19.0 (11.2 to 26.8)	18.1 (10.6 to 25.6)
	5	94.0 (89.3 to 98.7)	27.9 (19.1 to 36.6)	89.0 (82.8 to 95.2)	17.3 (9.9 to 24.7)	60.0 (50.2 to 69.8)	3.8 (0.1 to 7.6)

Abbreviations: CI, confidence interval; GMC, geometric mean concentration; MenC, monovalent group C meningococcal conjugate; Pnc9-MenC, 9-valent pneumococcal–group C meningococcal conjugate.
*At age 5 months for each putative serologic cutoff, proportion of Pnc9-MenC was greater than the corresponding MenC group proportion ($P<.001$ for all serotypes, by χ^2 test).

fects on Hib and MenC immunogenicity would be observed when used outside of the UK "accelerated" infant immunization schedule, which has only 1 month of separation between each dose, compared with 2 months of separation for US infants.

The effect of Pnc9-MenC on diphtheria immunogenicity is also unexpected. As a mutant diphtheria toxoid, the protein conjugate CRM_{197} used in both study vaccines can independently induce antibody against diphtheria.[19] The Pnc9–MenC vaccine contained nearly twice as much CRM_{197} (approximately 38.5 µg) compared with MenC (20 µg). At each of 3 immunization visits only 1 month apart, children received in addition to the study vaccines a further 30 IU of diphtheria as part of DTwP vaccine. However, the serum samples of all vaccinees exceeded the protective threshold of 0.01 IU/mL and the reduced diphtheria immunogenicity is probably of no clinical significance.

The sample size of this study was selected for the primary immunological end point of group C meningococcal SBA cutoff levels. As a result, the ability to detect adverse events associated with vaccination is limited only to common adverse events. The proportions of children with systemic reactions were similar to those expected following whole-cell pertussis–containing vaccines and were consistent with previously described rates post DTwP vaccination,[1] although irritability and decreased activity were more common after the third dose of Pnc9-MenC compared with MenC. In addition, more children in the Pnc9-MenC group were withdrawn for prolonged fever or crying (5 vs 1). Uncommon adverse events require ongoing cumulative monitoring across all clinical stages of development, including larger phase 3 trials, to enable associations to be detected. Rare associations may not be detected until postlicensure surveillance.

Although the study was randomized, the inability to blind clinical investigators and participating parents to vaccines given (due to visually different study vaccines) is a further limitation, with the potential for bias in reporting of adverse events. Laboratory staff performing immunological assays remained blinded to participant allocation. The high rate of refusal to participate, while typical of vaccine trials in developed countries, potentially diminishes the generalizability of these findings. Finally, we did not compare the combination vaccine with separate injections of MenC and Pnc9, as MenC is the standard currently used in the United Kingdom. It is possible that the administration of the vaccines separately may have had the same effect on immunogenicity to meningococcus or coadministered vaccine antigens as observed here when given as a combination vaccine. This possibility may warrant further study.

Since the pneumococcal serotype distribution associated with invasive disease varies around the world, increasing the number of serotypes in the pneumococcal conjugate vaccine is critical in generalizing its potential benefit to many nations, especially developing nations where most of the estimated 1.2 million childhood pneumococcal deaths occur each year.[20] Although the addition of serotypes 1 and 5 only increases serotype coverage for invasive bacteremic episodes from 82.2% to 82.6% in US children younger than 2 years,[21] in the United Kingdom this potential coverage is estimated to increase from 79.3% to 86.7% in children younger than 5 years when compared with the 7-valent vaccine.[22] In African nations where invasive isolates have been serotyped, adding serotypes 1 and 5 boosts coverage from 72% to 91% over that potentially offered by the 7-valent vaccine.[23]

These results highlight the unpredictability of immune responses to individual vaccine antigens after incorporating multiple antigens into combination vaccines and underline the importance of assessing the immunogenicity of all coadministered vaccine antigens in prelicensure trials. The Pnc9-MenC vaccine as tested may not be a suitable replacement for individual MenC or pneumococcal glycoconjugate vaccines.

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Acquisition of data: Buttery, Riddell, Chantler, Lane, Bowen-Morris, Morris, Harnden, Pollard.
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